Renewable Nylon through Commercialization of BIOLON™ DDDA
December 31, 2015

Primary Sponsor: Verdezyne, Inc.
2715 Loker Avenue West
Carlsbad, CA 92010
www.verdezyne.com

Contact Person: Thomas A. Beardslee, Ph.D.
Vice President, Research and Development
Verdezyne, Inc.
2715 Loker Avenue West
Carlsbad, CA 92010
760-707-5238
tbeardslee@verdezyne.com

Renewable Nylon through Commercialization of BIOLON™ DDDA

This nomination is eligible for the small business award.

This nomination is submitted for the focus area ***greener synthetic pathways***.

The integrated process for the production of renewable BIOLON™ DDDA described in this nomination was demonstrated at our 400-liter scale pilot facility throughout 2012 and 2013. Subsequent scale-up at external partner facilities in 2014 and 2015 enabled:
1) The production and sale of 70,000 lbs of renewable BIOLON™ DDDA,
2) Certification of our product with the USDA Certified Biobased product label,
3) The collection of data necessary for the engineering of our first commercial facility to be located in Malaysia, and
4) The signing of our first product distribution agreement with European specialty chemical distributor Will & Co., effectively securing off-take for over 25% of our plant's planned capacity.

The technology for this nomination was developed at Verdezyne's R&D headquarters and validated in our pilot plant, both located in Carlsbad, California. Further demonstration of the technology at larger scales was performed at partner facilities located in Michigan and Missouri.

Abstract: Verdezyne has developed a fermentation-based technology platform to provide manufacturers and consumers with renewable alternatives to existing petroleum-based chemical intermediates. The developed technology has focused on the production of the dicarboxylic acid chemical intermediates adipic acid, sebacic acid and dodecanedioic acid (DDDA). The first of these to be commercialized will be DDDA, which is used in many applications including coatings, corrosion inhibitors, adhesives, lubricants, and fragrances. However its main application is in the manufacture of nylon 6,12 for engineered plastics requiring special properties such as high chemical, moisture, or abrasion resistance.

The global market for DDDA is approximately 100 million pounds and is currently produced from fossil-based sources, with the largest volume technology proceeding via trimerization of butadiene followed by hydrogenation and oxidation with nitric acid. The Verdezyne process for production of DDDA uses fatty acid feedstocks sourced from the co-products of vegetable oil refining. In addition to providing a renewable alternative, our process offers improved safe operations without the need for high temperature and pressure or concentrated nitric acid, and offers reduced greenhouse gas emissions.

The technology for renewable DDDA has been developed at Verdezyne's R&D headquarters and with the 2011 commissioning of our pilot plant, the integrated process has been validated at pilot scale (400 L). Throughout 2014 and 2015, Verdezyne has demonstrated the process at larger scales (4,000 and 25,000 L), leading to the production and sale of 70,000 lbs of renewable BIOLON™ DDDA. This product met all industry quality specifications and has earned the USDA Certified Biobased product label, verifying that the product's amount of renewable biobased

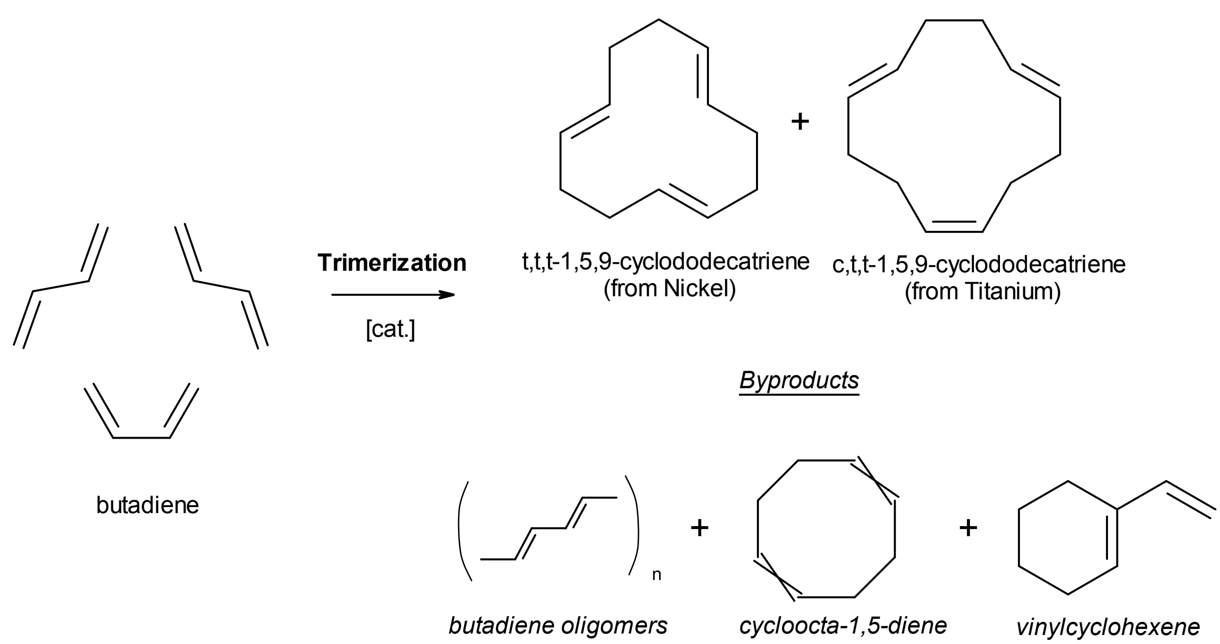
ingredients meets or exceeds levels set by the USDA. Our product is currently the only DDDA in the Biopreferred® catalog. We are currently preparing to break ground on a commercial-scale facility to be located in Nusajaya, Johor, Malaysia for the production of renewable BIOLON™ DDDA.

Renewable BIOLON™ DDDA as a Petrochemical Drop-in Replacement

Verdezyne has developed a robust industrial yeast strain and fermentation process capable of producing bio-based DDDA, a chemical that is currently produced from fossil-based sources. Our technology development has focused on producing renewable alternatives to existing petrochemicals where the fermentation product is the exact chemical currently used in the existing value chain, therefore making them drop-in replacements for petrochemicals. Producing these chemicals at or exceeding industry specifications allows manufacturers to switch to a renewable alternative without having to change equipment or procedures. This affords us the opportunity to take advantage of the existing markets for these chemicals for rapid commercialization.

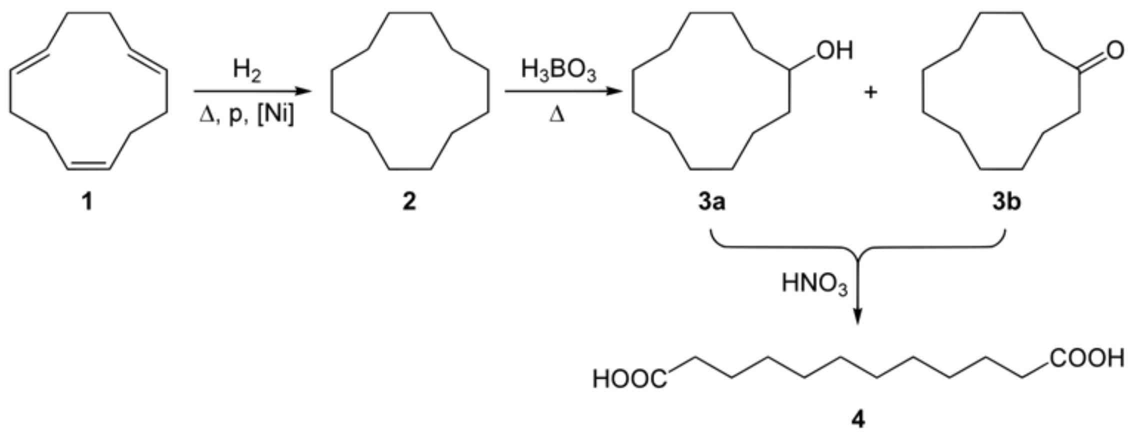
Two major producers, Invista and Cathay, supply the current world market for DDDA, which is approximately 100 million pounds. Specialty chemicals producers Evonik and UBE produce lower quantities, primarily for captive use. The Cathay process for the production of DDDA is fermentation-based, using dodecane purified by distillation from kerosene as the feedstock. The Invista process uses the petrochemical building-block molecule butadiene in a trimerization reaction (Equation 1) with a Ziegler-Natta titanium catalyst producing 1,5,9 cyclododecatriene

Equation 1: Synthesis of 1,5,9 cyclododecatriene from butadiene



(CDT) with a net selectivity of 90% for the desired *cis,trans,trans* isomer.[1] The CDT intermediate is then converted to DDDA in a multi-step process: catalytic hydrogenation at 170-180°C and 26-28 bar pressure absolute, mild oxidation with boric acid at 160-180°C resulting in a mixture of cyclododecanone and cyclododecanol, and ring-opening oxidation at 70-90°C with nitric acid and copper/vanadium catalyst (Equation 2).[2]

Equation 2: Synthesis of DDDA from 1,5,9 cyclododecatriene



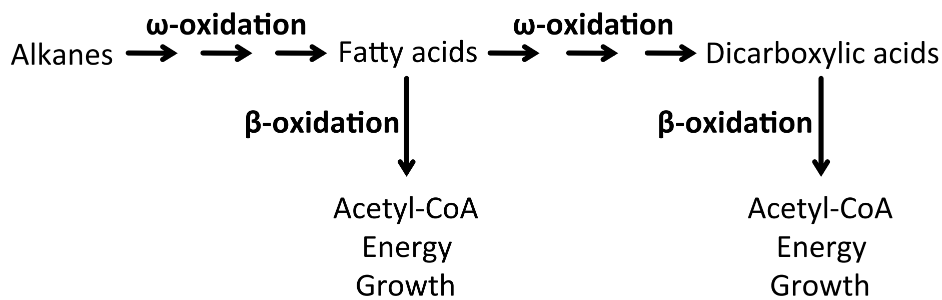
The last nitric acid oxidation step and the process for the production of nitric acid both release N_2O, a gas that has a global warming potential 298 times that of CO_2. The synthesis of adipic acid follows the same final reaction steps with six carbon molecules and releases ~0.3 tons of N_2O per ton of adipic acid produced in the nitric acid oxidation step.[3] Using the same stoichiometry, the final DDDA step would release ~0.2 tons of N_2O per ton of DDDA produced and consume ~0.5 tons of nitric acid per ton of DDDA produced. Abatement technologies for the destruction of both NO_x and N_2O in process emissions exist, however their effectiveness depends on the technology employed and the usage factor for the abatement at an individual facility. The amount of N_2O released to the atmosphere from current DDDA production is unknown. Nitric acid production is itself a significant source of greenhouse gas emissions with an estimated release of 36 kt of N_2O (10.7 million metric tons CO_2 equivalent) in 2013 from U.S. production facilities alone.[4]

The technology developed by Verdezyne is an improvement over the incumbent DDDA processes in that it: 1) reduces our dependence on petroleum by using a renewable feedstock, 2) operates under safer conditions with temperatures and pressures closer to ambient, and 3) eliminates the production of N_2O and the use of nitric acid.

Verdezyne's production of BIOLON™ DDDA is an aerobic fermentation process integrated with downstream product isolation and crystallization. The fermentation converts the twelve-carbon fatty acid, lauric acid, to DDDA through the activity of a genetically engineered version of *Candida* yeast. The natural lauric oils coconut oil and palm kernel oil (PKO), both have fatty acid compositions of approximately 50% lauric acid. PKO is the larger volume of the two with global production of ~10 billion pounds per year and finds its primary use in the oleochemical industry rather than in the food industry. Splitting of the triglyceride lauric oil into glycerol and fatty acids occurs through a number of technologies making lauric acid readily available as a free fatty acid or fatty acid ester.

The engineering of our yeast was enabled by our development of proprietary methods for its genetic manipulation and takes advantage of unique biochemistry native to the organism. The parental *Candida* yeast was originally isolated from petroleum contaminated soil due to its ability to use alkanes as its sole carbon source for growth. The unique biochemical pathway involved is the three-step ω-oxidation pathway that sequentially oxidizes the terminal end of an alkane (or a fatty acid) to a carboxylic acid. In the wild-type yeast, alkanes converted to fatty acids via ω-oxidation are consumed by the β-oxidation pathway generating the central two-carbon metabolite acetyl-CoA, energy, and allowing growth (Equation 3). Blocking the β-oxidation pathway by knocking out the first gene in the pathway results in an engineered strain that can no longer utilize alkanes or fatty acids as its sole carbon source for growth, however given a fatty acid and an energy source such as glucose the strain will produce dicarboxylic acids (diacids). Since the strain also cannot metabolize diacids the chain-length of the diacid product

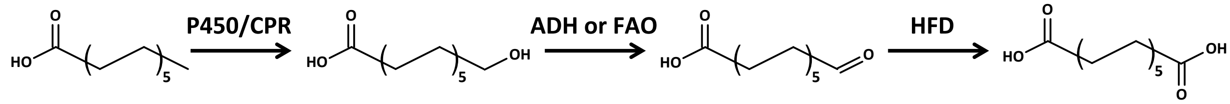
Equation 3: Biochemical pathways manipulated for BIOLON™ DDDA production



is the same as the chain length of the fatty acid entering ω-oxidation. Therefore, this type of β-oxidation blocked strain will produce DDDA from lauric acid however not at commercially relevant yields and productivities.

A commercial production yeast must be robust, efficient, and productive in converting feedstock to product. The Verdezyne BIOLON™ DDDA production host was specifically engineered for its task by amplifying the appropriate genes to enable rapid and high yield production of DDDA. Verdezyne holds the only known complete genome sequence for our production organism that allowed the identification of genes specifically involved in the conversion of lauric acid to DDDA. The amplification of these genes increase the overall yield and productivity of the process, and minimize the accumulation of pathway intermediates that can be toxic to the organism and detrimental to final product purity. The enzymes involved in the ω-oxidation pathway are shown in Equation 4. The key step for increasing overall flux through the pathway is the first step that is catalyzed by a two-enzyme system: a cytochrome P450 monoxoygenase (P450) that catalyzes the reaction and a cytochrome P450 reductase

Equation 4: Enzymes involved in the ω-oxidation pathway converting lauric acid to DDDA



(CPR) that regenerates the P450 for another round of oxidation. In our production yeast, a 10-gene family encodes P450 enzymes, with each gene encoding a P450 with different substrate specificity. Identification of the P450 enzyme specific for lauric acid and its subsequent amplification (along with other supporting gene amplifications) have resulted in our commercial production strain with yields in excess of 95% and rapid production of titers in excess of 140 g/L DDDA. The genetic engineering of our production host and the fermentation methods for diacid production are the subject of 4 issued patents and 33 pending patent applications. The technology for production of renewable DDDA from lauric acid feedstocks is the subject of patent application WO 2013/006730 A2.[5]

With the ultimate goal of building and operating a commercial facility for the production of renewable DDDA, we worked with partners to demonstrate the fermentation performance at larger scales. This demonstration was performed at the Michigan Biotechnology Institute, at the 4,000-liter scale and at ICM at the 25,000-liter scale. In both cases the fermentation performance was as good as or better in yield, productivity, and final titer than the performance seen at Verdezyne R&D and pilot facilities (Figure 1). DDDA was isolated during each campaign, resulting in the production of 70,000 lbs of finished product that met or exceeded industry specifications. All of this product was immediately sold and is currently being tested by potential future customers in their existing commercial applications. Reports received so far by Verdezyne are that BIOLON™ DDDA performs equal to or better than the petroleum based product in tested applications. In one application the report stated, "In side-by-side comparisons, Verdezyne's biobased DDDA yielded the highest-quality polymer that we have ever synthesized. Our end application is extremely sensitive to contaminants, which ultimately show up as undesirable colorants in the final product. By contrast, using Verdezyne's raw material we were able to produce the clearest, most functional material to date."[6] Our product has also earned the USDA Certified Biobased product label and is the only DDDA in the USDA Biopreferred® catalog.

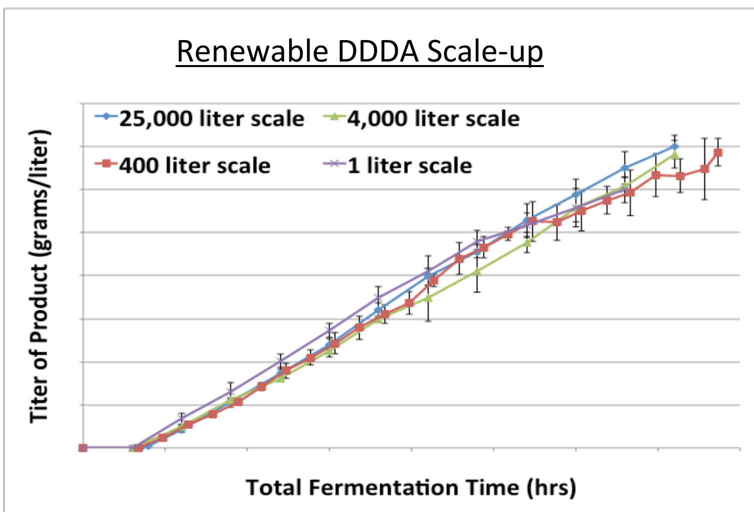


Figure 1: Equivalent fermentation performance at multiple scales

The data collected from these campaigns has also been used in the engineering of our commercial-scale facility for the production of BIOLON™ DDDA. The site selected for the 20

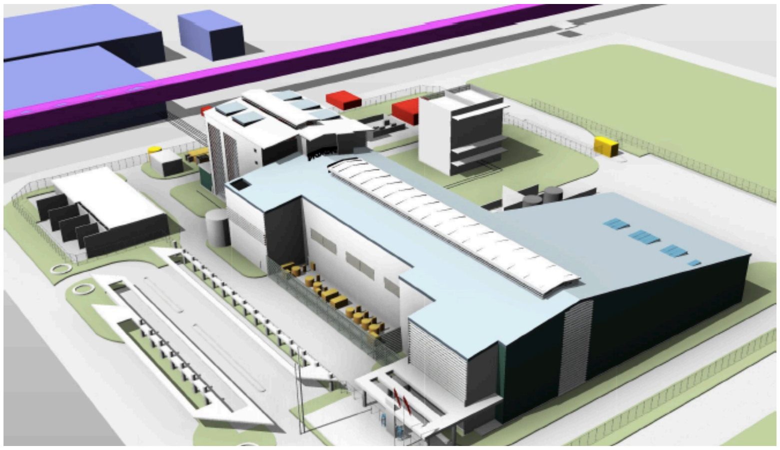


Figure 2: 3D rendering of planned commercial BIOLON™ DDDA facility

million pound facility (Figure 2) is the Bio-XCell bioindustrial park located in Nusajaya, Johor in southern peninsular Malaysia, just across the border from Singapore, strategically located close to the source of our lauric acid feedstock and to nearby ports for product distribution. The park provides existing infrastructure for security, park management, and a central utilities facility to provide electricity, steam, and wastewater treatment. We are currently in the detailed engineering phase for the project with groundbreaking planned for March 2016 and plant commissioning in late 2017. In compliance with local regulations stipulated by the Malaysian Ministry of Natural Resources and Environment, Verdezyne has officially registered our internal Institutional Biosafety Committee with the Malaysian Department of Biosafety and we have received approval from the Department of Biosafety for the contained use of our genetically modified yeast for production of DDDA.

As we near commercial production, we are putting in place the necessary supply chain, sales, and product distribution infrastructure and agreements. We recently reached a product distribution agreement with European specialty chemical distributor Will & Co, securing off-take for over 25% of the planned plant capacity.[7] Additional distribution agreements currently under negotiation could bring the total secured off-take to 50% of plant capacity. These agreements, likely to be completed well in advance of the completion of our commercial facility, reflect the surging global demand for biobased alternatives to petrochemicals. Additionally, the 100 million pound market for DDDA is growing at an estimated 5% annually with multiple applications, some of which are detailed below.

<u>Nylon:</u> Nylon 6,12 is based on DDDA and hexamethylenediamine. It is widely used in the manufacture of engineering resins and filaments. These engineering resins have lower moisture absorption and better dimensional stability compared with nylon 6,6 resins and are used in a variety of automotive applications. Flexibility imparted by the presence of DDDA makes nylon 6,12 filaments particularly useful in paintbrushes, toothbrushes, and cosmetic brushes. Nylon 12,12 containing DDDA and dodecanediamine has lower moisture absorption and greater flexibility than nylon 6,12, and is useful in high performance engineering resin applications, as well as in monofilament fishing line.

<u>Powder Coatings:</u> DDDA is widely used as a curing agent or cross-linker for glycidyl methacrylate (GMA) acrylic powder coatings. The use of DDDA in GMA acrylics results in coatings with better toughness, gloss, durability, chemical resistance, and flexibility. DDDA also

imparts good storage stability to powder coating compositions, in part because of its low solid/solid solubility in the bulk acrylic resin. DDDA linear polyanhydride and carboxy-terminated polyesters containing DDDA have also been used as curing agents for GMA acrylic powder coatings.

Lubricants and Greases: Diester lubricants based on DDDA have outstanding friction, wear and viscosity-temperature properties. Their performance is better than that of other shorter chain-length esters or hydrocarbon base oils, and have found applications in both automotive and aviation engine oils. Greases made with DDDA diesters also show bearing torque characteristics and oxidative stability superior to that of comparable greases made from a solvent neutral oil of similar viscosity.

Fragrances: C12 products are used in the manufacture of synthetic macrocyclic musks and also find applications in the synthesis of woody and floral notes. DDDA is used to produce a variety of macrocyclic ketones and lactones for the synthetic musk market.

The Verdezyne technology for production of BIOLON™ DDDA described above provides a renewable drop-in replacement for an existing petrochemical. In addition to reducing our dependence on finite petroleum resources contributing to global warming, the fermentation-based process can be carried out under safer operational conditions. We have successfully scaled-up our production process, verifying key metrics for competitive economics, and have demonstrated the highest product quality at each stage. Plans for our first commercial facility are moving steadily forward, and we are eagerly anticipating scaling up production to meet the world's surging demand for renewable DDDA and other chemicals.

---

[1] Zakharkin L and Guseva V (1978) The chemistry of 1,5,9-cyclododecatriene and syntheses based on it. Russian Chemical Reviews 47: 955-974.

[2] Rajendran G (2012) Process for the preparation of dodecanedioic acid. Subject of patent application EP2407444 A2.

[3] Reimer R, Slaten C, Seapan M, Koch T, and Triner V (2000) Adipic Acid Industry – N_2O Abatement. In "Non-CO2 Greenhouse Gases: Scientific Understanding, Control and Implementation", J. van Ham, et al. (eds.). Springer-Science+Business Media, B.V. p 347.

[4] Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2013. EPA 430-R-15-004. http://www3.epa.gov/climatechange/ghgemissions/usinventoryreport.html.

[5] Beardslee T, Picataggio S, Eirich ED, and Laplaza JM (2013) Biological methods for preparing a fatty dicarboxylic acid. Subject of patent application WO2013/006730 A2.

[6] Customer testimonial by Pete LeBaron, Ph.D., VP of Technology at ICM Products, April 21, 2015.

[7] http://verdezyne.com/2015/06/09/verdezyne-signs-agreement-with-major-european-chemicals-distributor-will-co/